REMARKS

Claim 1 has been amended by incorporating into the step the expression (a) "the annealing portion of the first DW-ACP is restricted to the portion consisting of said degenerated random nucleotide sequence and hybridizing nucleotide sequence at the first annealing temperature."

Claim 1 has been further amended to specify the number of the degenerate random sequence portion (Z_r) of the first degenerate DW-ACP is defined as from 2 to 5, and the language of "Y_q is continuously followed by Z_r" is incorporated.

Support for the above amendments can be found throughout the specification. Thus, no new matter is added into claim 1 by the amendments.

The preceding amendments and the following remarks are believed to be fully responsive to the outstanding Office Action and are believed to place the application in condition for allowance.

The Examiner is respectfully requested to reconsider and withdraw the rejections in view of the amendments and remarks as set forth hereinbelow.

Restriction Requirement

In a Restriction Requirement mailed May 5, 2008, the Examiner required a species election between the primers of claim 6, 8, and 12. Notwithstanding the election of a single species, Applicant is entitled to an initial search of the generic claim (in this case, claim 1). Claims to unelected species should only be withdrawn if the generic claim is finally held unallowable. As claim 1 remains under examination, Applicant respectfully requests consideration of all of the dependent claims irrespective of whether they read on the elected species.

Furthermore, upon further consideration of this case, it appears to Applicant that the initial requirement for a species election may have been made in error. In particular, claim 1 covers a method of amplification that requires the use of at least two and, in the case of dependent claim 9, three separate primers. Each of these primers has a distinct role in the unitary method of claim 1. In other words, the three primers do not represent three separate inventions but, rather, are each necessary components of a single invention. Therefore, Applicant respectfully submits that, for proper examination of this application, all three of the primers should be searched. Election of just one of the three claimed primers, as was required in this case, results in an incomplete search of the invention. While it may be appropriate for the Applicant select a species for **each** of the three claimed primers, a requirement for the election of

one of the three primers for examination is not. Applicant thus requests consideration and examination of all of the claims covering use of all of the primers used in the method of claim 1.

Rejections under 35 U.S.C. § 103(a)

Claim 1 was rejected for obviousness over Stone & Wharton (Nucleic Acids Research vol. 22, no. 13, pages 2612-2618, 1994), Welsh & McClelland (Nucleic Acids Research vol. 18, no. 24, pages 7213-7218, 1990), in view of Brenner (U.S. Patent No. 5,962,228), and further in view of Chun (WO 03/050305 A1).

At the outset, Applicant notes that, in interpreting claim 1, the Examiner states that the claims do not recite structural limitations of the claimed primers, because a specific template nucleic acid is not recited in the claims. However, the structure of the claimed first DW-ACP primer is clearly set forth in claim 1. Furthermore, because claim 1 is a method claim, the limitation that a region of the claimed first DW-ACP is complementary to a template sequence must be understood in the context of the overall method. Therefore, the claim covers a method of using a primer, where that primer contains a region complementary to a template sequence. Therefore, if a prior art sequence did not have a region complementary to a template, but otherwise resembled the claimed first DW-ACP, it would not render the claim obvious, because the operation of the claimed method requires a region of complementarity.

Applicant further notes that, in order to expedite prosecution, claim 1 has been amended by adding the language "the annealing portion of the first DW-ACP is restricted to the portion consisting of said degenerate random nucleotide sequence and hybridizing nucleotide sequence at the first annealing temperature." Applicant has further amended claim 1 so that the number of the nucleotide in the degenerate random sequence portion (Z_r portion) is 2-5, and the regulator portion (Y_q portion) is continuously followed by the degenerate random sequence portion (Z_r portion).

The Examiner states that the first amplification stage using the first degenerate DW-ACP of the instant claim 1 is taught by the arbitrary primed PCR method disclosed by Stone & Wharton, and Welsh and McClelland. However, the Applicant disagrees with the Examiner's opinion.

The principle of the arbitrary primed PCR (AP-PCR) is described in Welsh and McClelland (1990), page 7214, right column, lines 12 to 17 as below.

> Our rationale for the phenomenon of AP-PCR is as follows: At a sufficiently low temperature, primers can be expected to ***anneal to many sequences with a variety of mismatches.*** Some of these will be within a few hundred base pairs of each other and on opposite strands. Sequences between these positions will be PCR amplifiable. (Emphasis added).

As indicated in the above description, in the arbitrary primed PCR method, the temperature of low stringency condition is used for inducing a random mismatch between the primer sequence and the template sequence.

In contrast, the first DW-ACP of the instant invention specifically anneals to a template sequence that is complementary to a sequence of $Z_r + Q_s$ portion of it. The first DW-ACP of the present invention is fabricated so that Z_r portion comprises degenerate random sequences and Q_s portion include a hybridizing nucleotide sequence complementary to a site on unknown nucleotide sequence. So, if there is a nucleotide sequence in the template, which is complementary to $Z_r + Q_s$ portion of the first DW-ACP, specific hybridization can occur between $Z_r + Q_s$ portion in the first DW-ACP and the corresponding portion in the template.

As described in the paragraph of [0057] of the publication of the present application, "at the first annealing temperature, the annealing portion of the first DW-ACP is restricted to the degenerate sequence and 3'-end portions, thereby improving annealing specificity."

This feature is specified by amended claim 1 with the addition of the phrase "the annealing portion of the first DW-ACP is restricted to the portion consisting of said degenerated random nucleotide sequence and hybridizing nucleotide sequence at the first annealing temperature."

In summary, the first degenerate DW-ACP of the instant claim 1 can be specifically annealed to the target template through the specific hybridization between $Z_r + Q_s$ portion of the DW-ACP and the complementary portion in the template, resulting in the improvement of the reproducibility of the amplification, which is different and cannot be expected from the arbitrary primed PCR of the prior art.

Accordingly, the step (a-1) is of the instant claim 1 is not obvious from Stone & Wharton, and/or Welsh and McClelland.

Further, the Examiner states that Brenner teaches the process using the second DW-ACP of the present invention based on Brenner's disclosure of the use of rolling primer set containing complexity reducing nucleotides in primer walking approach.

Since the rolling primer taught by Brenner is used for a sequencing process, it targets one kind of template. Moreover, the rolling primer has to move by one nucleotide binding site on the same template at each cycle in the sequencing process. Thus, the rolling primer of Brenner must require a primer set consisting of multiple rolling primers because of its binding site shift. In order to minimize the number of rolling primers, Brenner uses complexity-reducing nucleotides. (see lines 1-3 of column 7 of Brenner, U.S. Patent No. 5,962,228).

In contrast, the claimed first degenerate DW-ACP having a degenerate random sequence is used in the first stage. Therefore, multiple templates having differing nucleotide sequences in the portion complementary to Z_r portion of the first degenerate DW-ACP are present at the step of second-stage amplification.

The region of the claimed second DW-ACP containing a universal base corresponds to the region in the multiple templates in which different nucleotides are incorporated. Therefore, the claimed second DW-ACP has the capacity to anneal to the resulting multiple templates despite only including a single sequence. In other words, even though it is a single primer, the regulation portion including a universal base enables the second DW-ACP to anneal to the respective multiple templates.

Based on this feature, the second claimed DW-ACP differs from the first degenerate DW-ACP. The second DW-ACP has a structure as defined in formula II (5'-X'_p-S_u-Y'_v-Z'_w-3'). The present claim 8 refers to the specific formula II of the second DW-ACP, which has been excluded from the examination as a non-elected species. However, as noted above, Applicant respectfully submits that examination of this claim is needed to obtain complete examination of the present invention.

The portion of S_u represents a supplementary annealing portion comprising a nucleotide sequence to hybridize with a portion opposite to the regulator portion of the first degenerate DW-ACP in the target-specific primer extension product of the step (a-2-1). The portion of Y'_v represents a regulator portion comprising at least two contiguous universal base or non-discriminatory base analog residues, and is able to hybridize with the portion corresponding to the degenerate random sequence of the first DW-ACP in the target-specific primer extension product of the step (a-2-1). The unique structure of the second DW-ACP is totally dependent on the distinctive structure of the first degenerate DW-ACP of the present invention.

Contrary to this, the general formula of the rolling primer of Brenner is expressed as $X_1X_2...X_kYY...$ YN (see column 7, line 56 of Brenner). The Y portion consists of not only universal base but also complements of the complexity-reducing nucleotides (see column 7, lines 59-60 of Brenner). In addition, as indicated in Figure 2b and column 5, lines 9-10 of Brenner, a

series of Y portions should be designed with complexity-reducing nucleotides (I) and their complements so that, the rolling primers containing the Y portions are used for the process of sequencing.

The second DW-ACP of the instant invention is represented as formula II of 5'-X'_p-S_u-Y'_v-Z'_w-3' as mentioned above. The 3'-end portion, Z'_w has a nucleotide sequence corresponding to the 3'-end portion of the first degenerate DW-ACP.

However, the 3'-terminal nucleotide of the rolling primer taught by Brenner is expressed as "N" and is either A, C, T, G or complexity-reducing nucleotide such as I. That is, the rolling primer set of Brenner comprises primers that contain different nucleotides in their 3'-end portion. The sequencing of the target template is carried out by observing what type of 3'-end nucleotide of the rolling primer set is extended. Please see the section of "Sequencing with Rolling Primers" from column 9, lines 60 to column 11, line 52, in particular column 10, lines 19-43.

Accordingly, the structure of 3'-end portion of the rolling primer taught by Brenner is different from that of the present second DW-ACP. There is a distinct structural difference in the 3'-end portion between the rolling primer of Brenner and the present second DW-ACP.

Hence, on the contrary to the opinion of the Examiner, the teachings of Stone & Wharton, Welsh & McClelland, and Brenner, as well as their combination, do not teach or suggest the structure and function of the second DW-ACP of the present method of claim 1.

The Examiner also states that Chun et al. (WO 03/050305) teaches the structure of the first degenerate DW-ACP of the present invention.

The Examiner indicates that Figure 5 of WO 03/050305 and, in particular, the 5' oligo in step 3 of the figure 5 teaches the structure of the claimed first degenerate DW-ACP. The 5' oligo in step 3 of figure 5 has the structure of '5'-preselected arbitrary sequence – regulator portion – arbitrary sequence – G-sequence.' In the Examiner's interpretation, the arbitrary sequence and G-sequence in figure 5 of WO 03/050305 corresponds to Z_r (the degenerate random portion) and Q_s (3'-end portion) of the claimed first degenerate DW-ACP respectively.

As mentioned above, the first degenerate DW-ACP of amended claim 1 specifies the number of nucleotides in the degenerate random sequence portion as 2-5, and the regulator portion (Y_q) is continuously followed by the degenerate random sequence portion (Z_r).

Example 6 of WO 03/050305 (page 94), in which the specific sequence of 5' oligo of figure 5 is described, explains that the arbitrary sequence of 5' oligo is the sequence of GGG, which is different from the claimed sequence of Z_r, the degenerate random portion of the first degenerate DW-ACP. As understood from figure 5, the arbitrary sequence of 5' oligo does not

have a complementary template, and thus, its sequence is not determined by a complementary template sequence but rather is selected from an artificial certain sequence.

In addition, Example 6 of WO 03/050305 describes that the G-sequence of the 3'-end of the 5' oligo is GGG, which is complementary to the CCC sequence resulting from the terminal transferase reaction of reverse transcriptase. Further, the nucleotide sequence of Q_s portion of the claimed first degenerate DW-ACP is capable of annealing to the specific nucleotide sequence of an unknown sequence. The nucleotide sequence of Q_s portion of the claimed first degenerate DW-ACP of this invention is different from that of the 3'-end of 5' oligo in figure 5 of WO 03/050305.

In summary, the specific structure of the claimed first degenerate DW-ACP is not disclosed in figure 5 of WO 03/050305.

The Examiner also indicates that claim 21 is rendered obvious by the JYC2-HD1 primer (represented as SEQ ID NO: 83) in Example 9 of WO 03/050305, which the Examiner contends teaches the structure of the claimed first degenerate DW-ACP.

The 3'-end portion nucleotide sequence, GT*N*C*RR*GTGTGGTT, of the JYC2-HD1 primer basically consists of a complementary sequence to a known, conserved nucleotide sequence of homeobox. The degenerate nucleotide *N* (A, T, C, or G) and *R* (A or G) in the above 3'-end portion sequence are incorporated in order to cover a variable nucleotide in the conserved nucleotide sequence. As a result, the conserved nucleotide sequence GT is positioned between the regulatory portion consisting of multiple 'I' residues and the 'N' nucleotide, and the conserved nucleotide sequence C is positioned between the degenerate nucleotides (N and RR), such as 'IIIII GT*N*C*RR*GTGTGGTT'. Therefore, the structure of JYC2-HD1 primer of SEQ ID NO: 83 can be expressed as Xp (5'-end portion) – Yq (universal base) - target specific nucleotides (GT) - degenerate nucleotide (N) - target specific nucleotide (C) – degenerate nucleotides (RR) – target specific nucleotides.

That is, the JYC2-HD1 primer (SEQ ID NO: 83) in Example 9 of WO 03/050305 employs R (A or G) in addition to N (A, C, G, or T) as a degenerate nucleotide. In contrast, the present invention uses only N as a degenerate nucleotide. In addition, the degenerate nucleotide is not present in a continuous form in the JYC2-HD1 primer but is interrupted with the target specific nucleotide of C. In contrast, amended claim 1 specifies that the degenerate nucleotide is present in a continuous form without interrupting nucleotides. Furthermore, the claimed first degenerate DW-ACP does not have any particular target specific nucleotide sequence located between Y_q (regulator portion of universal base) and Z_r (degenerate random sequence portion).

Also, the claimed number of contiguous degenerate nucleotides is from 2 to 5. This feature is not taught by the JYC2-HD1 primer (SEQ ID NO: 83) of WO 03/050305.

By incorporating Z_r (degenerate random sequence portion) between the Y_q (regulator portion of universal base) and Q_s (hybridizing nucleotide sequence 3'-end portion complementary to a site on unknown nucleotide sequence), the claimed first degenerate DW-ACP is more likely to hybridize to the unspecified target nucleic acid. Paragraph of [0040] of the publication of the present application, which describes this feature, is reproduced below.

> The term "degenerate random sequence" portion refers to a nucleotide sequence in which each nucleotide can be occupied by any one of the four deoxyribonucleotides, i.e., dATP, dTTP, dCTP, and dGTP. Thus, the degenerate random sequence portion provides a pool of primers with various nucleotide sequences, at least one of which will be anticipated to anneal to a site on an unknown target sequence of a template. For example, if the first degenerate DW-ACP comprising three degenerate nucleotides at the degenerate random sequence portion is synthesized, sixty-four distinct oligonucleotides are produced. Consequently, *the use of a degenerate random sequence portion provides the first degenerate DW-ACP with more probability to hybridize to the unspecified target nucleic acid.* When the target core sequence of the first degenerate DW-ACP including the degenerate sequence and 3'-end portion sequence hybridizes to a site of a template, there are several complementary binding sites of the degenerate portion and 3'-end portion sequence of the first DW-ACP present on the unknown target sequence of the template. (Emphasis added).

Further, the JYC2-HD1 primer (SEQ ID NO: 83) in Example 9 of WO 03/050305 is used for specific annealing to the known template sequence (a defined sequence based on the conserved sequence), while the claimed first degenerated DW-ACP is designed for specifically annealing to the unknown template sequence.

In summary, the specific structure of the claimed first degenerate DW-ACP is not taught or suggested by the JYC2-HD1 primer (SEQ ID NO: 83) of WO 03/050305.

In view of the above-described structural and functional differences between the present invention and the prior art, the method of amended claim 1 is not taught by a combination of the cited references. Consequently, the Applicant respectfully requests that the rejections for obviousness be withdrawn.

Applicants finally note that an Information Disclosure Statement and Form PTO 1449 were filed on April 1, 2009 and that an initialed copy of the 1449 has not been returned. Applicant request that such a copy be returned with the next action.

CONCLUSION

In view of the foregoing amendments and remarks, Applicant respectfully requests reconsideration and the timely allowance of the currently amended claims.

Transmitted herewith is a petition to extend the period for replying for three months, to and including January 27, 2010, and a Request for Continued Examination.

If there are any additional charges or any credits, please apply them to Deposit Account No. 03-2095.

Respectfully submitted,

Date: January 27, 2010

Susan M. Michaud

Susan M. Michaud, Ph.D.
Reg. No. 42,885

Clark & Elbing LLP
101 Federal Street
Boston, MA 02110
Telephone: 617-428-0200
Facsimile: 617-428-7045